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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.:	**DEX-0087**
Inventors:	**Recipon et al.**
Serial No.:	**09/705,500**
Filing Date:	**03 November 2000**
Examiner:	**Canella, Karen A.**
Group Art Unit:	**1642**
Title:	**A Novel Method of Diagnosing, Monitoring, Staging, Imaging and Treating Cancer**

Declaration by Dr. Roberto Macina

I, Roberto Macina, hereby declare:

1. I was awarded a M.S in Biology, and a Ph.D. in 1990 in Molecular Biology from the University of Buenos Aires, Argentina. After obtaining these degrees, I spent four years at The Wistar Institute, University of Pennsylvania contributing to the Human Genome Project endeavor. From 1995 to 1997, I served in the Molecular Diagnostic Department at SmithKline Beecham holding the positions of Investigator and Senior Investigator. Since the inception of diaDexus, Inc. in 1997 I have served as the Assistant Director of Cancer Gene Discovery. In October 2001 I assumed the position of Director of Molecular Technologies at diaDexus, Inc.

2. As the Director of Molecular Technologies for diaDexus, Inc., and a named inventor, I am familiar with the teachings of the above-referenced patent application.

3. The Gene Discovery division at diaDexus Inc. has performed experiments confirming the utility of Lng108 with regard to cancer.

4. Lng108 relative expression analysis was performed in accordance with a standard Quantitative Polymerase Chain Reaction (QPCR) protocol well known to those of skill in the art prior to November 1999, and outlined in the above-referenced patent application at page 20 line 16 through page 21 line 13.

5. I personally supervised experiments to measure the relative levels of Lng108 in cancerous, normal-adjacent, and normal tissues. In these experiments, relative quantitation of gene expression was done using Polymerase Chain Reaction in real time.

Quantitative PCR with fluorescent Taqman® probes is a quantitation detection system utilizing the 5′- 3′ nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman®) labeled with a 5′ reporter dye and a downstream, 3′ quencher dye. During PCR, the 5′-3′ nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA). Amplification of an endogenous control is used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase

(GAPDH), ATPase, or 18S ribosomal RNA (rRNA) is used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample were used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" can be obtained using the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

The tissue distribution and the level of mRNA expression of the target gene are evaluated for every sample in normal and cancer tissues. Total RNA is extracted from normal tissues, cancer tissues, and from cancers and the corresponding matched adjacent tissues. Subsequently, first strand cDNA is prepared with reverse transcriptase and the polymerase chain reaction is done using primers and Taqman® probes specific to each target gene. The results are analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of the target gene in a particular tissue compared to the calibrator tissue.

One of ordinary skill can design appropriate primers. The relative levels of expression of Lng108 versus normal tissues and other cancer tissues can then be determined. All the values are compared to the calibrator. Normal RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

The relative levels of expression of the Lng108 in pairs of matched samples may also be determined. A matched pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual. All the values are compared to the calibrator.

6. Results from this QPCR are depicted in the attached expression graphs. The expression graph shows that the levels of Lng108 are higher in the cancer samples tested when compared with all the normal tissues and the normal adjacent tissues for lung cancer. The sensitivity calculated comparing the levels of Lng108 in the lung cancer samples versus the expression in the lung normal adjacent tissue from the same patient is 43% (43% of the cancer samples show levels of Lng108 at least 2 fold higher than the corresponding normal adjacent form the same patient). The sensitivity calculated comparing the cancer samples versus the normal lung sample is of 19%. Thus, these experiments confirm the teachings of the above-referenced patent application of Lng108 being a cancer diagnostic marker.

7. Lng108 is at least as sensitive as many useful cancer therapeutics and diagnostics that have been FDA approved and are commercially available. For example, Genentech's product Herceptin® and its diagnostic counterpart, the HercepTest® are very successful commercially. Yet many publications show the relevant gene, HER-2, is overexpressed in 30% of breast cancer patients. Hence, in my professional opinion the sensitivity of Lng108 is sufficient for use with regard to cancer.

8. Methods for assessing whether a polynucleotide hybridizes under stringent conditions are well known to those of skill in the art and set forth in great detail in standard reference texts such as Sambrook et al. 1989 (Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor). Such methods can be performed routinely by those of skill in the art to

assess whether or not a Lng108 polynucleotide from a cell, tissue, or bodily fluid of a patient hybridizes under stringent conditions to an antisense sequence of SEQ ID NO: 1 or 2. In my professional opinion, a polynucleotide that hybridizes under stringent conditions to Lng108 could be readily identified without undue experimentation by one of ordinary skill in the art at the time of the invention.

9. In general, the relationship between mRNA expression levels and protein expression is well acknowledged and central to the dogma of molecular biology. Over-expression of mRNA and protein levels of many known and commercially successful cancer markers such as Herceptin® (HER-2) and Prostate Specific Antigen (PSA) are well documented. Based on my own experience, and numerous documented cases, the level mRNA expression correlates with protein levels. Hence, it is my professional opinion that the over-expression of Lng108 mRNA is sufficiently predictive of protein expressed by a polynucleotide of SEQ ID NO: 1 or 2.

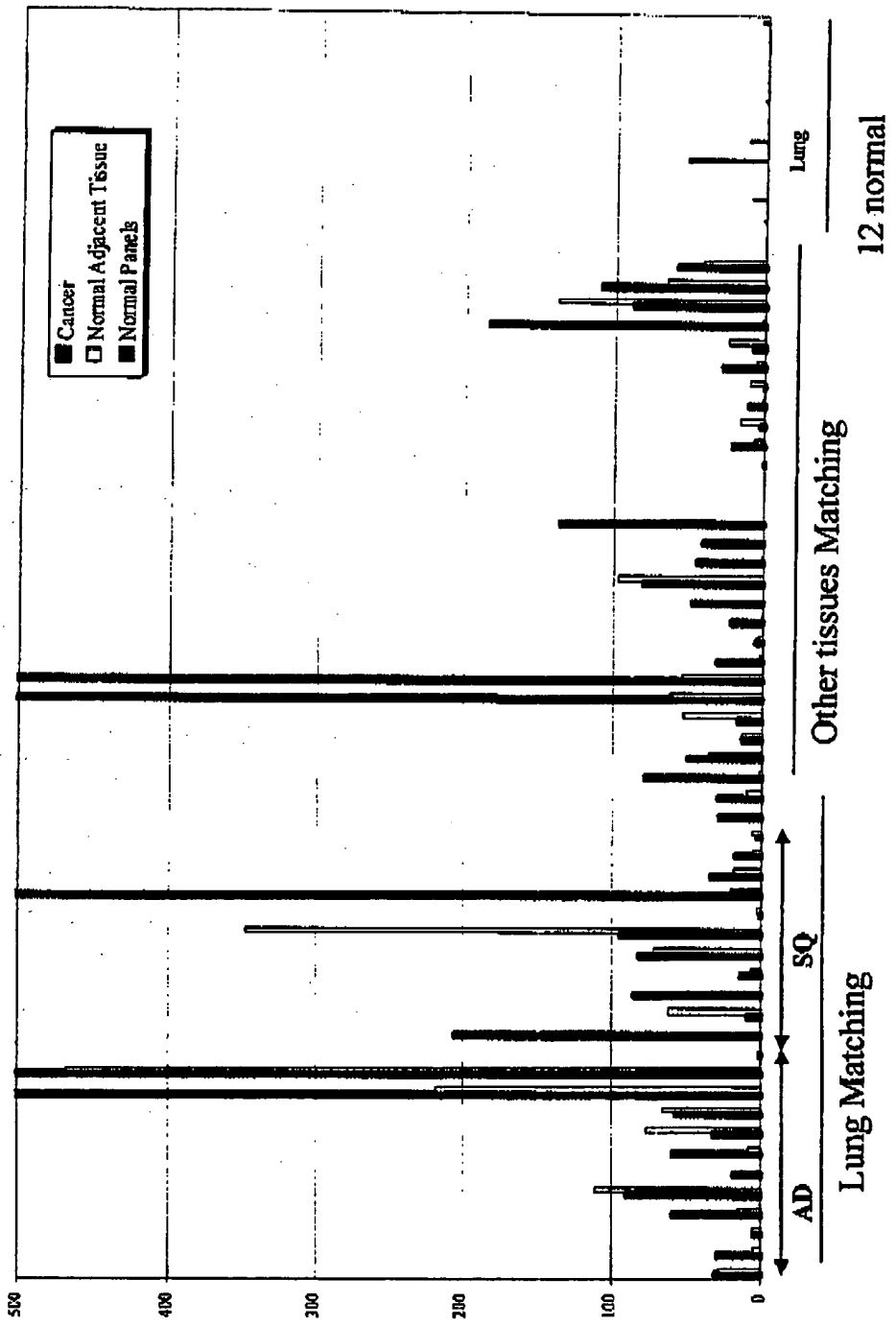
I hereby declare that all statements herein of my own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements were made with the knowledge that willful statements and the like so made are punishable by fine or by imprisonment, or both, under §1001 of Title 18 of the United States code, and that such willful statements may jeopardize the validity of the application, any patent issuing there upon, or any patent to which this verified statement is directed.

Roberto Macina, Ph.D.

03/24/03

Date

mRNA Expression for Lng108



mRNA Expression for Lng108